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Effect of acute cigarette withdrawal on cerebral blood flow and craving: a combined $H_2{}^{15}O$ PET study and self-report questionnaire

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To my parents,

SUMMARY Page

1 ABSTRACT

Objective

The purpose of this study was to investigate changes of cerebral blood flow and craving intensity in the acute phase of cigarette withdrawal.

Methods

Twenty male healthy smokers were recruited for the study. All were hospitalized for 4 days. Ten subjects (group A) stopped smoking on the morning of day 2 and 10 control subjects (group B) continued smoking.

$H_2{}^{15}O$ PET scanning for the measurement of regional and global cerebral blood flow (rCBF) was performed in both groups in the afternoon of day 1 and day 3. In each PET session, rCBF was measured 3 times and the mean was used for further analysis. For each measurement, a series of 18 frames of 10-seconds was initiated following the injection of 400-600 MBq $H_2{}^{15}O$. The arterial tracer concentration was measured in a catheter placed in the left radial artery. Quantitative maps of rCBF were calculated using Alpert's integral method.

The self-report Questionnaire on Smoking Urge Brief (QSU-Brief) was used to assess cigarette craving intensity. The QSU-Brief which consists of 10 items scored on a likert scale from 1 (I strongly agree) to 7 (I strongly disagree) was given to both groups at 8am, 11am, 2pm and 9pm from day 1 to day 3 and at 8am on day 4.

Craving intensity scores were calculated using the weighted mean on day 3 and day 1. Differences between treatments on day 3 were calculated by means of analysis of covariance, with day 1 taken as covariate.

Results

Regional CBF is indicated in ml/min/100gr. Baseline global gray matter blood flow (gmCBF) was 56.1 +/- 5.6 and 53.2 +/- 7.3 in group A and B respectively. In group A, gmCBF dropped to 48.0 (-14.3%, p=0.009, Students 2-tailed t-test) 32 hours following cigarette withdrawal. No significant change of gmCBF was noted in the control group. Regional analysis demonstrated that the decrease in group A was homogeneous in the whole brain with the exception of the cerebellum, where no significant change was noted.

On day 3 group A showed a significant higher craving score (3.8, SD=1.5) than Group B (2.8, SD=0.9) (p=0.01).

Conclusions

Acute cigarette withdrawal leads to a significant decrease of cerebral blood flow in all parts of the brain, except in the cerebellum. This is accompanied by an increase of subjective craving parameters. The absence of a CBF change in the cerebellum is possibly explained by absence of a nicotine-induced vasoactive mechanism. Craving changes are strongly significant despite of the artificial in-hospital condition. Relationship between CBF changes and nicotine withdrawal are discussed.

2 INTRODUCTION

2.1 Background

2.1.1 Cigarette Smoking

Cigarette smoking is known as the single major cause of avoidable morbidity and early mortality in developed countries[1]. Additionally, lung cancer is the largest single cause of cancer-associated mortality[2]. Nowadays, there are a worldwide-estimated 4.0 million deaths per year caused by tobacco[3], the figure being expected to rise to about 10 million in 2030. The risk of premature death due to tobacco is very high: half of all long-term smokers will eventually be killed by tobacco and half of these will die during the productive middle age, losing 20 to 25 years of life[4].

55 of the 4000 cigarette smoke chemicals have been evaluated by the International Agency for Research on Cancer (IARC) as showing "sufficient evidence for carcinogenicity" in either animals or humans. These substances include tobacco-specific nitrosamines (TSN), numerous polycyclic aromatic hydrocarbons (PAHs), radioactive polonium and benzene[5].

In 1997, Switzerland occupied one of the highest world prevalence rates, comparable only with those of Greece, Hungary and Poland, with about 38.4 % of men smoking among the Swiss population of more than 15 years and 27.4 % of the women. The cigarette consumption "per captia" is also declining slowly since 1970 (2^{nd} world rank) until 1992 (6^{th} world rank)[6]. From the 60'000 deaths declared each year in Switzerland, approximately 10'000 are attributed to tobacco.

2.1.2 Effects of Nicotine

Nicotine causes sympathetic stimulation by central and peripheral mechanisms. Its effects on the cardiovascular system are mediated by sympathetic neural stimulation, associated with an increase in the levels of circulating catecholamines, resulting in an acute increase in heart rate and blood pressure.

Nicotine affects blood flow to different organs, causing vasoconstriction in some vascular beds (i.e. skin) and vasodilatation in others (i.e. skeletal muscle).
Nicotine affects the metabolic rate; smokers weigh on average 4 kg less than non-smokers[7].
Nicotine has also a cholinergic effect responsible for central stimulation (attention, arousal and reaction time); nicotine relaxes the skeletal musculature, increases in the gastro-intestinal motility and secretion. It also produces memory facilitation, locomotor activation and anti-nociception[8].

2.1.3 Tiffany Questionnaire

Craving is the most prominent and bothersome symptom experienced during nicotine withdrawal and is anticipated by smokers as the most difficult aspect of quitting[9]. Tiffany and Drobes[10] (1991) developed the Questionnaire on Smoking Urges (QSU) to provide a more reliable measure and to assess the potential multidimensional nature of craving. Because of its length (32 items), the QSU is impractical for use. Therefore, an abbreviated questionnaire was developed to represent two of the four factors found in the longer QSU. The resulting QSU-Brief[11] contains 10 items and can be completed in less than 2 minutes (see Figure 1).

2.2 Nicotinic Acetylcholine Receptors (nAChRs)

2.2.1 Introduction

Nicotine acts as an agonist for nicotinic acetylcholine receptors. These nAChRs are expressed on mature skeletal muscle, in autonomic ganglia and within the central nervous system. Nicotine modulates the release of a number of neurotransmitters on presynaptic nAChRs and ganglionic potentials.

Neuronal nAChRs are members of the superfamily of ligand-gated ion channels neurotransmitter receptors. The neuronal nAChRs are composed of five membrane-spanning subunits (2α, 3β) that combine to form a functional receptor around a central pore or ion channel. Their distribution through the

brain is not restricted to the well defined cerebral cholinergic pathways. The majority of studies performed to date have mapped the distribution of AChR sites utilizing [^{3}H]-nicotine, [^{3}H]-epibatidine and [^{3}H]-cytisine. Highest levels of binding are observed in the thalamus, the nucleus basalis of Meynert (NBM) and the caudate nucleus. Moderate levels are seen in the frontal and parietal cortex, the putamen, the pons and the medulla; and relatively lower levels in the temporal and occipital cortex, the hippocampus and cerebellum.

2.2.2 Desensitization

The binding of 2 nicotine molecules on the 2α nAChR subunits causes an opening of the channel through an allosteric mechanism, which is selective for the cations Na^+, K^+. Then, the channel closes again and before to re-open, the receptor stays some milliseconds refractory to agonists. This refractory period lasts longer when nicotine stimulate the receptor, compared to the acetylcholine stimulation. In a smoker's brain, the nAChRs are over-stimulated by nicotine and as a result they enter progressively in an inactive state. This phenomenon is called desensitization[12]. In that way, nicotine acts as an antagonist, after chronic nicotine administration[13]. Olale (1997) proposed that the desensitization/inactivation of nAChRs following chronic nicotine exposure is the basis of tolerance to nicotine displayed by smokers[14].

2.2.3 Up-regulation

The nicotinic receptor shows a contradictory response to nicotine desensitization. It undergoes a paradoxical "up-regulation" in the presence of nicotine[15]. The receptor turnover reduces and the nAChR surface density increases[16].

In mice, chronic nicotine treatment induced a more pronounced percentage increase in [^{3}H]-nicotine binding in the cortex as compared with the mid-brain[17]. Human postmortem studies have revealed increased [^{3}H] nicotine and [^{3}H] ACh binding sites in the brains of smokers compared to non-smokers with a dose dependent correlation observed between increased binding sites and the number of cigarettes smoked[18]. Some studies have found a significant level of nicotinic receptors in brain samples from smokers compared with nonsmokers in the gyrus rectus of the frontal lobe, the hippocampus, the cerebellum and the thalamus[19].

2.3 Cerebral Blood Flow (CBF)

2.3.1 Regulation of CBF

The cerebral vessels are innervated by nerve fibers emanating from cell bodies in ganglia belonging to the sympathetic, parasympathetic and sensory nervous systems. In addition, cerebral resistance vessels may be innervated by nerves that originate within the brain which represent an intrinsic nerve supply[20].

The regulation of cerebral blood flow is complex, only partially known, involving a multitude of mechanisms and molecules (noradrenalin, neuropeptide Y, Serotonin, VIP, nitric oxide and carbon dioxide). CO_2 is one of the most potent endogenous modulators of CBF and acts as a cerebral vasodilator. Under normal circumstances, hypercapnia increases CBF and hypocapnia decreases CBF.

2.3.2 Acute Administration of Nicotine and CBF

Non-invasive ultrasonic Doppler velocimetry to measure blood flow in cerebral arteries has shown that acute smoking increases blood flow in the middle cerebral artery, internal carotid artery and vertebral artery[21].

Sinkhoj et al.[22] (1973) measured CBF, with the Xenon-133 intracarotid injection technique, in 23 patients after they had smoked a cigarette. Smoking increased gray matter blood flow by 15.7 %, without any regional variations.

Nagata et al. (1997) studied nicotine administration in a sample of smokers. Increases in rCBF after smoking were observed in the orbitofrontal region and nucleus accumbens, with reductions in the occipital cortex.

With functional-MRI the effects of intravenous nicotine have also been studied by Stein et al.[23] (1998). Nicotine increased rCBF in the cingulated and prefrontal cortices, portions of the occipital and temporal cortices, nucleus accumbens, amygdala, insular cortex, thalamus and hypothalamus.

Domino et al.[24] (2001) investigated the effect of nasal nicotine spray on CBF in 11 overnight abstinent habitual smokers with $H_2{}^{15}O$ PET. Nicotine spray and a placebo were administered in a single-blind design. Regional CBF

increases were noted in the frontal, the cingulate and occipito-temporal cortices, the cuneus and the thalamus. Nicotine reduced rCBF in the left insula and in the occipital cortex.

Lee et al.[25] proposed a model explaining the molecular mechanism of neurogenic vasodilation of cerebral arteries under nicotine stimulation. Next to a large porcine artery from the base of the brain, nicotine acts on presynaptic nicotinic receptors located on the adrenergic nerve terminals to release norepinephrine (NE), which then acted on the presynaptic β_2-adrenoceptors located on the neighboring nitric oxidergic (NOergic) nerve terminals to release NO, therefore induces vasodilation.

Overall, the current consensus is that both acute smoking and acute nicotine administration increase CBF.

2.3.3 Long term effects of Nicotine on CBF

Cigarette is a well known risk factor for stroke. The long-term changes observed in CBF are thought to be the result of atherosclerosis, or disturbance of the endothelial cell function produced by nicotine. Kubota et al.[26] (1983), using the xenon 133 inhalation technique, found significantly lower CBF (-12%) in 37 smokers than in 74 matched non-smokers. Rogers et al. (1985) with the same technique observed also a lower CBF (-10%) in 71 current smokers, compared with 140 non-smokers.

To our knowledge, the rCBF in smokers at a high craving state has not yet been evaluated previously. We hypothesized that acute nicotine withdrawal would reduce the global CBF, as acute nicotine administration increases the global CBF.

3 MATERIAL-METHODS

3.1 Subjects

The study was approved by the local ethical committee and all subjects gave written informed consent. Twenty two healthy male smokers with no history of psychiatric, neurological or other medical illness were recruited for this study. One of them had to be dismissed because no arterial line could be placed. In a second one there was a technical PET problem. Thus this study was performed on 20 healthy male smokers. The subjects were right-handed and at least 35 years old. They did not intend to give up (Fagerström Questionnaire score ≥7; see Figure 2) and were free from current use of any nicotine replacement therapy. They had a body mass index within the range 19-29 $kg.m^{-2}$, no history of alcohol or drug abuse, no history of drug allergy and had exhaled carbon monoxide (CO) >8ppm at baseline.

They were divided in two groups; group A: 10 smokers, who had free access to cigarettes during day 1 and enforced smoke withdrawal during day 2 and 3; group B: 10 continual smokers, who had free access to cigarettes during day 1, 2 and 3. The hospitalization period was 4 days.

The subject characteristics are presented in table 1. The mean subject was 44 years old, smoked one and a half pack of cigarette a day (since 26 years), drank 4 coffees and one alcohol unit per day.

3.2 Study Design

3.2.1 Methods

This is an open, randomized, parallel study, on two groups.
The subjects were required to refrain from all medication, both prescribed and over the counter, for two weeks prior to enrolment until the end of the study. They received the same diet during the confinement period. Caffeine

intake over the 12 hours prior to the baseline scan was similar to that consumed on the day of dosing.

Group A (Enforced Cessation Smokers): subjects were required to smoke freely during day 1 upon entry to the unit and to stop smoking on the morning of day 2 (08:00 am). Smoking cessation was maintained until the morning of day 4 (08:00 am). PET scans were performed in the afternoon of day 1 (free smoke period) and in the afternoon of day 3 (enforced smoking cessation period). A baseline MRI scan was recorded during subjects' staying in the unit, to aid with regional brain mapping. The timing was at discretion of the investigator. Subjects were discharged from the investigation unit on the morning of day 4 (12:00 am), provided the investigator was satisfied with their medical condition.

Group B (Continual Smokers): subjects were required to smoke freely upon entry to the unit. PET scans were performed in the afternoon of day 1 and in the afternoon of day 3. A baseline MRI scan was recorded during subjects' staying in the unit. Subjects were discharged from the investigation unit on the morning of day 4 (12:00 am), provided the investigator was satisfied with their medical condition.

3.2.2 Recorded Parameters

Smoked Cigarettes and Exhaled CO

Daily number of cigarettes during free smoking periods was recorded.
CO was assessed at screening visit, from day 1 to day 3 at 8am, 11am, 2pm, 9pm and on the morning of day 4 at 8am (plus the pre PET assessments: just before PET). PET and MR scanning were performed between 4 and 7pm on day 1 and day 3.

Craving assessments

All subjects were also administered the TSFQ (Tiffany Short Form Questionnaire or QSU-Brief) to evaluate the craving intensity during the study period. The TQSF was administered at the following time points:

Group A (Enforced Cessation Smokers): from day 1 to day 3: at 8am, 11am, 2pm and 9pm; at 8am, 2pm and 9pm the TSFQ was soon after the meal. In

the morning of day 2, subjects were smoking their last cigarette 0-30 minutes before the TSFQ administration.

Group B (Continual Smokers): from day 1 to day 3: at 8am, 11am, 2pm and 9pm; at 8am, 2pm and 9pm the TSFQ was soon after the meal. In the morning of day 1 to 3, subjects had to smoke their 1st cigarette 0-30 minutes before the TSFQ administration.

Arterial CO_2 and Blood Pressure

Immediately before each injection of $H_2{}^{15}O$ arterial CO_2 was determined. Additionally blood pressure was measured immediately after each PET measurement.

3.2.3 Positron Emission Tomography (PET)

PET utilizes compounds labeled with short-lived, positron-emitting radionuclides. These tracer compounds are injected intravenously and are distributed, bound and eliminated according to the biological properties of the native compound. After a positron has been emitted from the tracer, it annihilates and produces two high-energy gamma rays that are readily detected by the surrounding detector system. The raw data thus collected are reconstructed and a time series of tomographic images are generated. The images show the quantitative distribution and time course of tracer in field of view.

Water can be readily labeled with positron emitting ^{15}O. The half-life of ^{15}O is just 2 minutes. Following intravenous injection tissue accumulation of $H_2{}^{15}O$ is used as a marker of tissue perfusion. In this study, $H_2{}^{15}O$ was used to assess regional changes in regional cerebral blood flow (rCBF).

3.3 Imaging

Positron emission tomography; data acquisition and calculation of rCBF maps

The PET studies were performed on a whole body scanner (Advance, GE Medical Systems, Milwaukee, WI, U.S.A.) with a 14.6-cm axial field of view

and a 7-mm reconstructed in-plane resolution. Before positioning of the patients in the scanner, catheters were placed in an antecubital vein for tracer injection and the radial artery for blood sampling. Three studies were performed within 45 minutes. Between the perfusion studies, a 10-minute transmission scan was acquired for the correction of photon attenuation. For each perfusion study, 400 to 600 MBq $H_2{}^{15}O$ was injected using an automatic injection device, which delivers a predefined amount of activity over 20 seconds. With the arrival of the bolus to the brain, a series of 18 scans of 10 seconds each was initiated in three-dimensional mode. Continuous sampling of arterial blood drawn from the radial artery assessed the time course of the arterial radioactivity.

Transaxial images of the brain were reconstructed using filtered backprojection (128×128 matrix, 35 slices, 2.34×2.34 ×4.25-mm voxel size). Quantitative parametric maps representing rCBF were calculated using the integration method described by Alpert et al.[27] (1984). This method represents a computationally efficient implementation of the flow calculations based on the time course of the activity concentration in each voxel and arterial blood (input curve). It is based on the solution of the one-tissue compartment model for $H_2{}^{15}O$. The operational equation is:

$$C(t) = K_1\ C_a(t) \otimes e^{-k_2 t} = K_1 \int_0^t C_a(t) e^{k_2 (u-t)} du$$

where $C(t)$ represents tissue activity, $C_a(t)$ is the measured arterial input function (AIF) and k is rCBF/p (flow/partition coefficient). Essentially, a lookup table r is calculated from the blood data as follows:

$$r = \frac{\int_0^T \int_0^t C_a(u) e^{k_2 (u-t)} du dt}{\int_0^T t \int_0^t C_a(u) e^{k_2 (u-t)} du dt}$$

where T denotes the acquisition duration and k is varied in 400 increments between 0 and 200 (ml/min/100gr) to cover the range of k occurring in

physiologic conditions. A similar operation is performed with the PET data in each voxel:

$$\hat{r} = \frac{\int_0^T \int_0^t C(u)\,du\,dt}{\int_0^T t \int_0^t C(u)\,du\,dt}$$

From the actual k is obtained by a lookup in the r table. The flow is finally calculated by entering k into Eq. 4:

$$rCBF = K_1 = \frac{\int_0^T \int_0^t C(u)\,du\,dt}{\int_0^T t \int_0^t C_a(u) e^{k_2(u-t)}\,du\,dt}$$

Compared to the true input curve in the brain the measured time course of arterial activity in the radial artery is time-shifted and distorted due to dispersion. Both time shift and dispersion were corrected before calculation of the rCBF by the method described by Meyer[28] (1989). To this end, a flow model including the delay and an exponential dispersion as parameters was fitted to the averaged time-activity curve of all brain pixels with integrated activity above 40% of the maximum. The measured AIF was then shifted by the estimated delay and deconvolved by the exponentially decaying dispersion using a Fourier transform approach. All computations were performed with the dedicated JAVA-based software PMOD (http://www.pmod.com) by Mikolajczyk et al.[29] (1998), which allows the easy implementation of the needed models to calculate rCBF, including the corrections of the input curve.

Data analysis

The multiple PET rCBF flow maps per imaging session were first averaged. The resulting mean map was then transformed into stereotactic space using the software SPM99. In a next step the mean rCBF of gray matter ($rCBF_{gray}$) was determined the following way; a mask of gray matter was constructed by

using a threshold of 80% of the maximum brain value. All voxels below that threshold were removed. The mask of gray matter voxels was then applied to all rCBF maps in stereotactic space and the mean of all voxels was calculated ($rCBF_{gray}$). The difference of $rCBF_{gray}$ on day3 and day1 was then calculated and compared for the 2 groups A and B using Student's 2-tailed t-test.

In order to look for areas that showed a potentially localized deviation in the day 1-day 3 difference from the whole brain mean statistical parametric mapping (SPM) was performed. In a first step rCBF maps were normalized to the mean value of gray matter. In this way all global differences in whole brain CBF were removed. After this normalization the scans were smoothed with a 15 mm Gaussian filter and SPM performed using the software SPM99. The analysis was done for group A and group B separately.

The regional blood flow was examined with independent repeated-measures analyses of variance (ANOVA) for the global blood flow and the cerebellum blood flow, where ROI (region of interest) was the dependent variable and the group (A and B), the independent variable. The hypothesis with no ROI effect and with no ROI*Group effect was tested, as well as the group effect on ROI.

4 RESULTS

Figure 3 illustrates the activity's time course of the 18 10-seconds frames in the brain; the represented slice cuts the brain immediately above the thalamus. From the start of the tracer injection, the delay lasts about 20 seconds until activity is detected in the brain. In fact, every points represent corrected values.

Continuous sampling of arterial blood drawn from the radial artery assessed the time-course of the arterial tissue radioactivity. This is represented by the blue curve on Figure 4. The corrected PET counts are drawn in pink. Thus, the PET counter detects the first activity at the third frame (20 seconds delay), whereas the arterial blood sampling counter perceives it 10-15 seconds later, because of the longer distance (~2x) for the blood to arrive to the arterial counter. We measured the arterial activity until 30 seconds after the end of the scan.

Figure 5 represents an example of a quantitative flow map from a $H_2{}^{15}O$ PET. The red spots correspond to regions with a high regional CBF. For example, on the two first slices (up and left), the petrosal part of the two internal carotid arteries can be recognized.

The difference of $rCBF_{gray}$ on day 3 and day 1 is demonstrated in Table 2. There was a 14 % decrease of $rCBF_{gray}$ (8.047 ml/min/100gr; SD=4.88) in the group that stopped smoking and only a minimal change in the group that continued smoking (0.967 ml/min/100gr; SD=3.46). The difference between the groups was highly significant (p=0.00015).

Table 2 illustrates also the significant difference ($p<0.001$) of the expired CO before the PET on day 1 and 3. Obviously the group who stopped smoking had a very significant decrease of CO values (–90,36 %; SD=18.84). However, the group who continued smoking showed an 11.03 % decrease of CO values (SD=5.46). Compared to stable CO values, this CO decrease is not significant (p=0.1).

$PaCO_2$ was measured before every scan. On day 3 the group A showed slight $paCO_2$ decrease (–3.35 %; SD=5.46). In contrast group B showed out a minimal decrease of $paCO_2$ (–1.10 %; SD=6.40). These minimal changes were not significant (p=0.59).

The blood pressure was measured after every scan. The variation of systolic blood pressure between day 1 and day 3 was in group A +0.6 mmHg (SD=9.39) and –0.2 mmHg (SD=6.39) in group B. On the other hand, the diastolic values of the blood pressure showed variations of +0.7 mmHg (SD=8.02) for the group A and of –2.5 mmHg (SD=4,86) for the group B. These changes in the blood pressures were only minimal and not significant (systolic BP p=0.65; diastolic BP p=0.12).

The craving intensity was measured with the Tiffany questionnaire (QSU-Brief), before the PET on day 1 and day 3. The total urge score was calculated by the mean of the 10 items. Figure 6 represents the difference of craving intensities on day 3 compared with day 1 (baseline). Group A showed a significant increase on day 3 (+1.35 points; SD=1.24) and group B demonstrated a slight decrease (–0.38 points; SD=0.75).

The result of statistical parametric mapping (SPM) searching for excessive changes in rCBF is shown in Figure 7 and Figure 8.
Figure 7 demonstrates areas that show an excessive decrease of blood flow from day 1 to day 3, that is where the decrease is significantly (T>5) higher than the general decrease of 14 %. The analysis reveals one area in the left insular cortex. A ROI analysis demonstrated that the decrease in this area was -19.4 % (Figure 7). The color spots at the surface of the brain correspond to artifacts.
Figure 6 reveals areas that show the smallest gray matter decrease with T>5 from day 1 to day 3 in the group that stopped smoking. The analysis identified the cerebellum. The ROI analysis demonstrates that the change in the cerebellum was only -2.9 % (Figure 8).

Figure 9 summarizes the changes in regional cerebral blood flow. First is represented a global gray matter CBF decrease in the group who stopped smoking and a minor CBF increase in the group who continued to smoke. In group A, all the analyzed regions (blue) illustrated a drop of rCBF. There was

a clear discrepancy between the cerebellum (–2.5 %) that showed no significant change between day 1 and day 3 (p=0.61) and the other investigated areas, which all demonstrate a significant decrease (range: –9.3 % to –19.6 %). In group B, no significant regional difference was found between day 1 and day 3.

Effects of experimental conditions were explored with repeated-measures analyses of variance (ANOVA) for the global blood flow and the cerebellum. The hypothesis with no ROI effect was significant $F_{(1,18)}$=7.43; p=0.014, as well as the hypothesis with no ROI*Group effect $F_{(1,18)}$=4.63; p=0.045. The repeated measure ANOVAs demonstrated an effect of experimental condition, when compared between group A and B and Day 1 and 3, on the global blood flow $F_{(1,18)}$=22.67; p=0.0002, but not on the cerebellar blood flow $F_{(1,18)}$=0.81; p=0.379.

5 DISCUSSION

The major result of this study is the demonstration of a significant decrease of rCBF in smokers who interrupt nicotine intake. The decrease seems to be more or less homogeneous in the gray matter of the neocortex and is in the order of 14%. Almost no change is noted in the cerebellum. The control parameters ($paCO_2$ and blood pressure) did not show any significant variations for both groups, while the parameters controlling the smoking cessation (CO and QSU-Brief) showed a significant change for group A, but not for group B. The 11% decrease of CO values in the control group could be explained by the in-hospital condition and by the simultaneous presence of another volunteer who often took place in the non-smoking group (according to our randomization table).

The CBF decrease seen in our nicotine withdrawn subjects could correspond to the interruption of the vasodilative effect of nicotine. Every time that a smoker smokes, his plasma nicotine concentration and his CBF increase. Every time that a smoker does not smoke, his plasma nicotine concentration and his CBF slowly decrease, until the next cigarette. In the situation of total abstinence, the CBF would return to original values, but under the toxic action of nicotine on the arteries, the CBF of smokers progressively decrease over the years (~10%). Consequently the CBF values of the nicotine withdrawn smoker in the acute phase lays under the CBF values of the non-smoker and under the values of the smoking smoker. These lower values lasts months to years, until the ex-smoker partially recovers his original CBF.

Previous studies

Volkow[30] (1999) reported a study where no CBF change was found after 4 hours of smoking cessation (short-term abstinence) and another one with a decrease of rCBF in the hippocampus bilaterally (after 4 to 6 hours of nicotine deprivation). The aim of these 2 studies was to demonstrate a difference between the rCBF in smokers, compared with non-smokers and primarily not the craving effects. Although the subject can endure some withdrawal symptoms after 4 to 6 hours smoking cessation, these 2 studies did not show a clear demonstration of a complete nicotine withdrawal syndrome.

Conversely, 2 studies evaluated the CBF of long-term abstinent smokers. Rogers et al.[31] (1985) showed in 11 abstinent smokers (from 1 to 12 months of smoking abstinence) a significant CBF recovery of 1 ml/min/100gr every month. Yamashita et al.[32] (2000) showed in six smokers an improvement of the rCBF, only after 4 to 6 years of tobacco interruption. After months of smoking abstinence, craving mostly persists and the CBF return slowly to his original values.

To our knowledge, there is no study evaluating the rCBF in smokers at a high craving state (32 hours smoking abstinence in our study). At this time, the nicotine withdrawal somatic symptoms are present and the relapse rate is high.

5.1 The nAChR hypothesis

In a normal brain the nAChR density is high in some regions (thalamus, caudate nucleus, frontal and parietal cortex) and low in temporal and occipital cortex, hippocampus and cerebellum.

In the particular case of the cigarette smoker, the global density of nAChR is higher than in a non-smoker's brain (up-regulation). Secondly they are mostly in a desensitized or inactive state. Human post-mortem studies have found a significantly higher density of nicotinic receptors labeled by [^{3}H]-nicotine in brain samples from smokers compared with non-smokers in the gyrus rectus of the frontal lobe, the hippocampus, the cerebellum and the thalamus[33].

To understand the density variation of the nAChR in a brain during nicotine withdrawal syndrome, Pietilä et al. (1998)[34] showed in mice, chronically treated with nicotine that the receptor density decrease and return to control level 7 days after nicotine withdrawal.
The up-regulation of nicotinic receptors in mice has previously been reported by Collins et al.[35] (1988) to return to control levels within 6–8 days after cessation of chronic nicotine treatment. In rats, the up-regulation of the

nAChR returns to normal level after 15-20 days after nicotine cessation. (Collins 1990).
In 2 baboons after chronic nicotine treatment, Kassiou et al.[36] (2001) demonstrated a full nAChR down-regulation in 3 regions (frontal lobe, thalamus and cerebellum) after 5 weeks nicotine withdrawal.
In human brains, Breese et al.[37] (1997) demonstrated in thalamus and hippocampus the recovery of the nAChR up-regulation by smokers, who stopped smoking at least 2 months prior to death. The other gray matter regions were not analyzed.
Even with the lack of data in the literature concerning the other gray matter regions of the human brain, we could postulate that in these, the up-regulation recovery lasts at least some weeks. It seems improbable that this nAChR down-regulation after nicotine cessation could explain the CBF decrease seen in our subjects, because they were enduring a nicotine withdrawal of 36 hours long. But during that time, the desensitized nAChRs partially re-sensitize. This re-sensitization of the nAChR can lead to an excess of receptive receptors, but without the agonist nicotine. This hyper-excitability state at cholinergic synapses plays probably a role in the development of the craving symptoms. The question remains if this hyper-excitability plays a role in the CBF decrease of our subjects.

5.2 The NBM hypothesis

Some studies on animals demonstrated the participation of the nucleus basalis of Meynert (NBM) to cause a nicotine-induced cholinergic neuronal vasodilation. Meynert described the anatomy of these magnocellular neurons at the base of the brain in 1872. The nucleus basalis is a part of the basal forebrain, which is a group of structures (NBM, septal area and the diagonal band of Broca) that are just rostral to the diencephalon. The basal forebrain projects dense cholinergic pathways to the surrounding structures, as well as the NBM which projects also to most regions of the neocortex.

Uchida and Sato (1997) measured the CBF responses to an intravenous nicotine administration in rats under different conditions. Some had the NBM electrically injured on both sides, some received mecamylamine (MEC), a

blood brain barrier permeable nicotinic receptors antagonist. Others were given atropine (muscarinic cholinergic antagonist) or hexamethonium. The nicotine-induced CBF increase was largely reduced by these three nicotine antagonists. The rats with NBM lesions had comparable CBF reductions. The authors concluded that the nicotine-induced cortical vasodilation was mediated by activation of the nicotinic receptors in the NBM[38].

In addition Linville et al. (1991, 1993)[39,40] provoked CBF increases in rats by electrical micro-stimulation of the basal forebrain. They also demonstrated that nicotine microinjections into the basal forebrain elicited profound increases in cortical CBF, whereas similar injections into the cerebral cortex were without effect suggesting that nicotine receptors mediating CBF increases are localized to the basal forebrain.

Nicotine is thought--at least in rats--to increase the CBF partially through the activation of the neurons situated in the NBM, projecting in most part of the cortex, but not to the cerebellum; the remaining part of activation is composed by local metabolites and by others projecting neurons.

In the human brain, the role of the NBM under nicotine and under a nicotine withdrawal situation is still unclear. But if the human NBM functions like the NBM of the rat, we could suggest that with every cigarette, nicotine stimulates the NBM producing a CBF increase as long as the nicotine plasma levels are high. At nicotine withdrawal, the nAChR of the NBM are not anymore activated and thus the CBF decrease.

5.3 Other Considerations

Cerebellum

The cerebellum of our subjects, withdrawn from nicotine did not show any significant CBF changes, in comparison of the gray matter of the neocortex. The cerebellum has in contrast with other gray matter brain areas, a lower nAChR density and a higher subunit polymorphism. Some authors reported a significant nAChR up-regulation in the cerebellum after chronic nicotine treatment in humans, but not in mice.

As the cerebellum of our subjects has also up-regulated nAChRs, we should have observed a similar CBF decrease between the cerebellum and the neocortex. This makes the nAChRs hypothesis unlikely.
In opposition, the absence of a cholinergic innervation from the nucleus basalis of Meynert to the cerebellum[41,42] could explain the lack of CBF decrease observed in the cerebellum.
In addition, the cerebellum reacts under an acute nicotine administration with a CBF decrease, as reported by Ghatan et al (1998) and Nagata et al. (1998). This might be explained by a steal-effect from the cerebellum to the neocortex, when the latter increase his CBF under acute nicotine administration.

Left Insula

Once the global change in rCBF was established, statistical parametric mapping SPM99 was used to search for focal areas that might deviate from the global range. This efficient tool revealed one small focus in the left insular area where the decrease of rCBF significantly exceeded the mean decrease (14%) of whole brain gray matter. In this area, the decrease was approximately 19%.
Projections from the NMB to the insular cortex have been documented, as well as from the insular cortex to the NMB[43]. However the physiological meaning of this finding remains at present not clear.

6 CONCLUSION

To our knowledge, this is the first study evaluating the rCBF of smokers in the acute phase of nicotine withdrawal. The major finding of this study is a 14% decrease of CBF in the gray matter, except in the cerebellum where no significant change was noted.

During nicotine withdrawal by smokers, it has been demonstrated that down-regulation of up-regulated nAChRs lasts weeks or months, indicating that nicotinic receptor density is probably not the factor involved in the CBF decrease.

Moreover, the forebrain contains a neuronal cholinergic pathway, originating from the nucleus basalis of Meynert and projecting to the majority of the regions of the cortex. Its cholinergic activation is known to produce a significant CBF increase in the rat. One may therefore speculate, that a deactivation of the NBM responsible for the decrease in CBF shown in this study. This is further supported by the fact that no change was observed in the cerebellum, which do not received projections from the NBM.

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Effect of acute cigarette withdrawal on cerebral blood flow and craving: a combined $H_2{}^{15}O$ PET study and self-report questionnaire

INAUGURAL-DISSERTATION
zur Erlangung der Doktorwürde der Medizinischen Fakultät
der Universität Zürich

vorgelegt von
Mathieu JOBIN
von les Bois JU

Genehmigt auf Antrag von Prof. Dr. med. et dipl. Masch. ing. ETH A. Buck
Zürich 2003

To my parents,

SUMMARY Page

1 ABSTRACT

Objective

The purpose of this study was to investigate changes of cerebral blood flow and craving intensity in the acute phase of cigarette withdrawal.

Methods

Twenty male healthy smokers were recruited for the study. All were hospitalized for 4 days. Ten subjects (group A) stopped smoking on the morning of day 2 and 10 control subjects (group B) continued smoking.

$H_2{}^{15}O$ PET scanning for the measurement of regional and global cerebral blood flow (rCBF) was performed in both groups in the afternoon of day 1 and day 3. In each PET session, rCBF was measured 3 times and the mean was used for further analysis. For each measurement, a series of 18 frames of 10-seconds was initiated following the injection of 400-600 MBq $H_2{}^{15}O$. The arterial tracer concentration was measured in a catheter placed in the left radial artery. Quantitative maps of rCBF were calculated using Alpert's integral method.

The self-report Questionnaire on Smoking Urge Brief (QSU-Brief) was used to assess cigarette craving intensity. The QSU-Brief which consists of 10 items scored on a likert scale from 1 (I strongly agree) to 7 (I strongly disagree) was given to both groups at 8am, 11am, 2pm and 9pm from day 1 to day 3 and at 8am on day 4.

Craving intensity scores were calculated using the weighted mean on day 3 and day 1. Differences between treatments on day 3 were calculated by means of analysis of covariance, with day 1 taken as covariate.

Results

Regional CBF is indicated in ml/min/100gr. Baseline global gray matter blood flow (gmCBF) was 56.1 +/- 5.6 and 53.2 +/- 7.3 in group A and B respectively. In group A, gmCBF dropped to 48.0 (-14.3%, p=0.009, Students 2-tailed t-test) 32 hours following cigarette withdrawal. No significant change of gmCBF was noted in the control group. Regional analysis demonstrated that the decrease in group A was homogeneous in the whole brain with the exception of the cerebellum, where no significant change was noted.

On day 3 group A showed a significant higher craving score (3.8, SD=1.5) than Group B (2.8, SD=0.9) (p=0.01).

Conclusions

Acute cigarette withdrawal leads to a significant decrease of cerebral blood flow in all parts of the brain, except in the cerebellum. This is accompanied by an increase of subjective craving parameters. The absence of a CBF change in the cerebellum is possibly explained by absence of a nicotine-induced vasoactive mechanism. Craving changes are strongly significant despite of the artificial in-hospital condition. Relationship between CBF changes and nicotine withdrawal are discussed.

2 INTRODUCTION

2.1 Background

2.1.1 Cigarette Smoking

Cigarette smoking is known as the single major cause of avoidable morbidity and early mortality in developed countries[1]. Additionally, lung cancer is the largest single cause of cancer-associated mortality[2]. Nowadays, there are a worldwide-estimated 4.0 million deaths per year caused by tobacco[3], the figure being expected to rise to about 10 million in 2030. The risk of premature death due to tobacco is very high: half of all long-term smokers will eventually be killed by tobacco and half of these will die during the productive middle age, losing 20 to 25 years of life[4].

55 of the 4000 cigarette smoke chemicals have been evaluated by the International Agency for Research on Cancer (IARC) as showing "sufficient evidence for carcinogenicity" in either animals or humans. These substances include tobacco-specific nitrosamines (TSN), numerous polycyclic aromatic hydrocarbons (PAHs), radioactive polonium and benzene[5].

In 1997, Switzerland occupied one of the highest world prevalence rates, comparable only with those of Greece, Hungary and Poland, with about 38.4 % of men smoking among the Swiss population of more than 15 years and 27.4 % of the women. The cigarette consumption "per captia" is also declining slowly since 1970 (2^{nd} world rank) until 1992 (6^{th} world rank)[6]. From the 60'000 deaths declared each year in Switzerland, approximately 10'000 are attributed to tobacco.

2.1.2 Effects of Nicotine

Nicotine causes sympathetic stimulation by central and peripheral mechanisms. Its effects on the cardiovascular system are mediated by sympathetic neural stimulation, associated with an increase in the levels of circulating catecholamines, resulting in an acute increase in heart rate and blood pressure.

Nicotine affects blood flow to different organs, causing vasoconstriction in some vascular beds (i.e. skin) and vasodilatation in others (i.e. skeletal muscle).
Nicotine affects the metabolic rate; smokers weigh on average 4 kg less than non-smokers[7].
Nicotine has also a cholinergic effect responsible for central stimulation (attention, arousal and reaction time); nicotine relaxes the skeletal musculature, increases in the gastro-intestinal motility and secretion. It also produces memory facilitation, locomotor activation and anti-nociception[8].

2.1.3 Tiffany Questionnaire

Craving is the most prominent and bothersome symptom experienced during nicotine withdrawal and is anticipated by smokers as the most difficult aspect of quitting[9]. Tiffany and Drobes[10] (1991) developed the Questionnaire on Smoking Urges (QSU) to provide a more reliable measure and to assess the potential multidimensional nature of craving. Because of its length (32 items), the QSU is impractical for use. Therefore, an abbreviated questionnaire was developed to represent two of the four factors found in the longer QSU. The resulting QSU-Brief[11] contains 10 items and can be completed in less than 2 minutes (see Figure 1).

2.2 Nicotinic Acetylcholine Receptors (nAChRs)

2.2.1 Introduction

Nicotine acts as an agonist for nicotinic acetylcholine receptors. These nAChRs are expressed on mature skeletal muscle, in autonomic ganglia and within the central nervous system. Nicotine modulates the release of a number of neurotransmitters on presynaptic nAChRs and ganglionic potentials.

Neuronal nAChRs are members of the superfamily of ligand-gated ion channels neurotransmitter receptors. The neuronal nAChRs are composed of five membrane-spanning subunits (2α, 3β) that combine to form a functional receptor around a central pore or ion channel. Their distribution through the

brain is not restricted to the well defined cerebral cholinergic pathways. The majority of studies performed to date have mapped the distribution of AChR sites utilizing [^{3}H]-nicotine, [^{3}H]-epibatidine and [^{3}H]-cytisine. Highest levels of binding are observed in the thalamus, the nucleus basalis of Meynert (NBM) and the caudate nucleus. Moderate levels are seen in the frontal and parietal cortex, the putamen, the pons and the medulla; and relatively lower levels in the temporal and occipital cortex, the hippocampus and cerebellum.

2.2.2 Desensitization

The binding of 2 nicotine molecules on the 2α nAChR subunits causes an opening of the channel through an allosteric mechanism, which is selective for the cations Na^+, K^+. Then, the channel closes again and before to re-open, the receptor stays some milliseconds refractory to agonists. This refractory period lasts longer when nicotine stimulate the receptor, compared to the acetylcholine stimulation. In a smoker's brain, the nAChRs are over-stimulated by nicotine and as a result they enter progressively in an inactive state. This phenomenon is called desensitization[12]. In that way, nicotine acts as an antagonist, after chronic nicotine administration[13]. Olale (1997) proposed that the desensitization/inactivation of nAChRs following chronic nicotine exposure is the basis of tolerance to nicotine displayed by smokers[14].

2.2.3 Up-regulation

The nicotinic receptor shows a contradictory response to nicotine desensitization. It undergoes a paradoxical "up-regulation" in the presence of nicotine[15]. The receptor turnover reduces and the nAChR surface density increases[16].

In mice, chronic nicotine treatment induced a more pronounced percentage increase in [^{3}H]-nicotine binding in the cortex as compared with the mid-brain[17]. Human postmortem studies have revealed increased [^{3}H] nicotine and [^{3}H] ACh binding sites in the brains of smokers compared to non-smokers with a dose dependent correlation observed between increased binding sites and the number of cigarettes smoked[18]. Some studies have found a significant level of nicotinic receptors in brain samples from smokers compared with nonsmokers in the gyrus rectus of the frontal lobe, the hippocampus, the cerebellum and the thalamus[19].

2.3 Cerebral Blood Flow (CBF)

2.3.1 Regulation of CBF

The cerebral vessels are innervated by nerve fibers emanating from cell bodies in ganglia belonging to the sympathetic, parasympathetic and sensory nervous systems. In addition, cerebral resistance vessels may be innervated by nerves that originate within the brain which represent an intrinsic nerve supply[20].

The regulation of cerebral blood flow is complex, only partially known, involving a multitude of mechanisms and molecules (noradrenalin, neuropeptide Y, Serotonin, VIP, nitric oxide and carbon dioxide). CO_2 is one of the most potent endogenous modulators of CBF and acts as a cerebral vasodilator. Under normal circumstances, hypercapnia increases CBF and hypocapnia decreases CBF.

2.3.2 Acute Administration of Nicotine and CBF

Non-invasive ultrasonic Doppler velocimetry to measure blood flow in cerebral arteries has shown that acute smoking increases blood flow in the middle cerebral artery, internal carotid artery and vertebral artery[21].

Sinkhoj et al.[22] (1973) measured CBF, with the Xenon-133 intracarotid injection technique, in 23 patients after they had smoked a cigarette. Smoking increased gray matter blood flow by 15.7 %, without any regional variations.

Nagata et al. (1997) studied nicotine administration in a sample of smokers. Increases in rCBF after smoking were observed in the orbitofrontal region and nucleus accumbens, with reductions in the occipital cortex.

With functional-MRI the effects of intravenous nicotine have also been studied by Stein et al.[23] (1998). Nicotine increased rCBF in the cingulated and prefrontal cortices, portions of the occipital and temporal cortices, nucleus accumbens, amygdala, insular cortex, thalamus and hypothalamus.

Domino et al.[24] (2001) investigated the effect of nasal nicotine spray on CBF in 11 overnight abstinent habitual smokers with $H_2{}^{15}O$ PET. Nicotine spray and a placebo were administered in a single-blind design. Regional CBF

increases were noted in the frontal, the cingulate and occipito-temporal cortices, the cuneus and the thalamus. Nicotine reduced rCBF in the left insula and in the occipital cortex.

Lee et al.[25] proposed a model explaining the molecular mechanism of neurogenic vasodilation of cerebral arteries under nicotine stimulation. Next to a large porcine artery from the base of the brain, nicotine acts on presynaptic nicotinic receptors located on the adrenergic nerve terminals to release norepinephrine (NE), which then acted on the presynaptic β_2-adrenoceptors located on the neighboring nitric oxidergic (NOergic) nerve terminals to release NO, therefore induces vasodilation.

Overall, the current consensus is that both acute smoking and acute nicotine administration increase CBF.

2.3.3 Long term effects of Nicotine on CBF

Cigarette is a well known risk factor for stroke. The long-term changes observed in CBF are thought to be the result of atherosclerosis, or disturbance of the endothelial cell function produced by nicotine. Kubota et al.[26] (1983), using the xenon 133 inhalation technique, found significantly lower CBF (-12%) in 37 smokers than in 74 matched non-smokers. Rogers et al. (1985) with the same technique observed also a lower CBF (-10%) in 71 current smokers, compared with 140 non-smokers.

To our knowledge, the rCBF in smokers at a high craving state has not yet been evaluated previously. We hypothesized that acute nicotine withdrawal would reduce the global CBF, as acute nicotine administration increases the global CBF.

3 MATERIAL-METHODS

3.1 Subjects

The study was approved by the local ethical committee and all subjects gave written informed consent. Twenty two healthy male smokers with no history of psychiatric, neurological or other medical illness were recruited for this study. One of them had to be dismissed because no arterial line could be placed. In a second one there was a technical PET problem. Thus this study was performed on 20 healthy male smokers. The subjects were right-handed and at least 35 years old. They did not intend to give up (Fagerström Questionnaire score ≥7; see Figure 2) and were free from current use of any nicotine replacement therapy. They had a body mass index within the range 19-29 $kg.m^{-2}$, no history of alcohol or drug abuse, no history of drug allergy and had exhaled carbon monoxide (CO) >8ppm at baseline.

They were divided in two groups; group A: 10 smokers, who had free access to cigarettes during day 1 and enforced smoke withdrawal during day 2 and 3; group B: 10 continual smokers, who had free access to cigarettes during day 1, 2 and 3. The hospitalization period was 4 days.

The subject characteristics are presented in table 1. The mean subject was 44 years old, smoked one and a half pack of cigarette a day (since 26 years), drank 4 coffees and one alcohol unit per day.

3.2 Study Design

3.2.1 Methods

This is an open, randomized, parallel study, on two groups.
The subjects were required to refrain from all medication, both prescribed and over the counter, for two weeks prior to enrolment until the end of the study. They received the same diet during the confinement period. Caffeine

intake over the 12 hours prior to the baseline scan was similar to that consumed on the day of dosing.

Group A (Enforced Cessation Smokers): subjects were required to smoke freely during day 1 upon entry to the unit and to stop smoking on the morning of day 2 (08:00 am). Smoking cessation was maintained until the morning of day 4 (08:00 am). PET scans were performed in the afternoon of day 1 (free smoke period) and in the afternoon of day 3 (enforced smoking cessation period). A baseline MRI scan was recorded during subjects' staying in the unit, to aid with regional brain mapping. The timing was at discretion of the investigator. Subjects were discharged from the investigation unit on the morning of day 4 (12:00 am), provided the investigator was satisfied with their medical condition.

Group B (Continual Smokers): subjects were required to smoke freely upon entry to the unit. PET scans were performed in the afternoon of day 1 and in the afternoon of day 3. A baseline MRI scan was recorded during subjects' staying in the unit. Subjects were discharged from the investigation unit on the morning of day 4 (12:00 am), provided the investigator was satisfied with their medical condition.

3.2.2 Recorded Parameters

Smoked Cigarettes and Exhaled CO

Daily number of cigarettes during free smoking periods was recorded.
CO was assessed at screening visit, from day 1 to day 3 at 8am, 11am, 2pm, 9pm and on the morning of day 4 at 8am (plus the pre PET assessments: just before PET). PET and MR scanning were performed between 4 and 7pm on day 1 and day 3.

Craving assessments

All subjects were also administered the TSFQ (Tiffany Short Form Questionnaire or QSU-Brief) to evaluate the craving intensity during the study period. The TQSF was administered at the following time points:

Group A (Enforced Cessation Smokers): from day 1 to day 3: at 8am, 11am, 2pm and 9pm; at 8am, 2pm and 9pm the TSFQ was soon after the meal. In

the morning of day 2, subjects were smoking their last cigarette 0-30 minutes before the TSFQ administration.

Group B (Continual Smokers): from day 1 to day 3: at 8am, 11am, 2pm and 9pm; at 8am, 2pm and 9pm the TSFQ was soon after the meal. In the morning of day 1 to 3, subjects had to smoke their 1st cigarette 0-30 minutes before the TSFQ administration.

Arterial CO_2 and Blood Pressure

Immediately before each injection of $H_2{}^{15}O$ arterial CO_2 was determined. Additionally blood pressure was measured immediately after each PET measurement.

3.2.3 Positron Emission Tomography (PET)

PET utilizes compounds labeled with short-lived, positron-emitting radionuclides. These tracer compounds are injected intravenously and are distributed, bound and eliminated according to the biological properties of the native compound. After a positron has been emitted from the tracer, it annihilates and produces two high-energy gamma rays that are readily detected by the surrounding detector system. The raw data thus collected are reconstructed and a time series of tomographic images are generated. The images show the quantitative distribution and time course of tracer in field of view.

Water can be readily labeled with positron emitting ^{15}O. The half-life of ^{15}O is just 2 minutes. Following intravenous injection tissue accumulation of $H_2{}^{15}O$ is used as a marker of tissue perfusion. In this study, $H_2{}^{15}O$ was used to assess regional changes in regional cerebral blood flow (rCBF).

3.3 Imaging

Positron emission tomography; data acquisition and calculation of rCBF maps

The PET studies were performed on a whole body scanner (Advance, GE Medical Systems, Milwaukee, WI, U.S.A.) with a 14.6-cm axial field of view

and a 7-mm reconstructed in-plane resolution. Before positioning of the patients in the scanner, catheters were placed in an antecubital vein for tracer injection and the radial artery for blood sampling. Three studies were performed within 45 minutes. Between the perfusion studies, a 10-minute transmission scan was acquired for the correction of photon attenuation. For each perfusion study, 400 to 600 MBq $H_2{}^{15}O$ was injected using an automatic injection device, which delivers a predefined amount of activity over 20 seconds. With the arrival of the bolus to the brain, a series of 18 scans of 10 seconds each was initiated in three-dimensional mode. Continuous sampling of arterial blood drawn from the radial artery assessed the time course of the arterial radioactivity.

Transaxial images of the brain were reconstructed using filtered backprojection (128×128 matrix, 35 slices, 2.34×2.34 ×4.25-mm voxel size). Quantitative parametric maps representing rCBF were calculated using the integration method described by Alpert et al.[27] (1984). This method represents a computationally efficient implementation of the flow calculations based on the time course of the activity concentration in each voxel and arterial blood (input curve). It is based on the solution of the one-tissue compartment model for $H_2{}^{15}O$. The operational equation is:

$$C(t) = K_1\ C_a(t) \otimes e^{-k_2 t} = K_1 \int_0^t C_a(t) e^{k_2 (u-t)} du$$

where $C(t)$ represents tissue activity, $C_a(t)$ is the measured arterial input function (AIF) and k is rCBF/p (flow/partition coefficient). Essentially, a lookup table r is calculated from the blood data as follows:

$$r = \frac{\int_0^T \int_0^t C_a(u) e^{k_2 (u-t)} du dt}{\int_0^T t \int_0^t C_a(u) e^{k_2 (u-t)} du dt}$$

where T denotes the acquisition duration and k is varied in 400 increments between 0 and 200 (ml/min/100gr) to cover the range of k occurring in

physiologic conditions. A similar operation is performed with the PET data in each voxel:

$$\hat{r} = \frac{\int_0^T \int_0^t C(u)\,du\,dt}{\int_0^T t \int_0^t C(u)\,du\,dt}$$

From the actual k is obtained by a lookup in the r table. The flow is finally calculated by entering k into Eq. 4:

$$rCBF = K_1 = \frac{\int_0^T \int_0^t C(u)\,du\,dt}{\int_0^T t \int_0^t C_a(u) e^{k_2(u-t)}\,du\,dt}$$

Compared to the true input curve in the brain the measured time course of arterial activity in the radial artery is time-shifted and distorted due to dispersion. Both time shift and dispersion were corrected before calculation of the rCBF by the method described by Meyer[28] (1989). To this end, a flow model including the delay and an exponential dispersion as parameters was fitted to the averaged time-activity curve of all brain pixels with integrated activity above 40% of the maximum. The measured AIF was then shifted by the estimated delay and deconvolved by the exponentially decaying dispersion using a Fourier transform approach. All computations were performed with the dedicated JAVA-based software PMOD (http://www.pmod.com) by Mikolajczyk et al.[29] (1998), which allows the easy implementation of the needed models to calculate rCBF, including the corrections of the input curve.

Data analysis

The multiple PET rCBF flow maps per imaging session were first averaged. The resulting mean map was then transformed into stereotactic space using the software SPM99. In a next step the mean rCBF of gray matter ($rCBF_{gray}$) was determined the following way; a mask of gray matter was constructed by

using a threshold of 80% of the maximum brain value. All voxels below that threshold were removed. The mask of gray matter voxels was then applied to all rCBF maps in stereotactic space and the mean of all voxels was calculated ($rCBF_{gray}$). The difference of $rCBF_{gray}$ on day3 and day1 was then calculated and compared for the 2 groups A and B using Student's 2-tailed t-test.

In order to look for areas that showed a potentially localized deviation in the day 1-day 3 difference from the whole brain mean statistical parametric mapping (SPM) was performed. In a first step rCBF maps were normalized to the mean value of gray matter. In this way all global differences in whole brain CBF were removed. After this normalization the scans were smoothed with a 15 mm Gaussian filter and SPM performed using the software SPM99. The analysis was done for group A and group B separately.

The regional blood flow was examined with independent repeated-measures analyses of variance (ANOVA) for the global blood flow and the cerebellum blood flow, where ROI (region of interest) was the dependent variable and the group (A and B), the independent variable. The hypothesis with no ROI effect and with no ROI*Group effect was tested, as well as the group effect on ROI.

4 RESULTS

Figure 3 illustrates the activity's time course of the 18 10-seconds frames in the brain; the represented slice cuts the brain immediately above the thalamus. From the start of the tracer injection, the delay lasts about 20 seconds until activity is detected in the brain. In fact, every points represent corrected values.

Continuous sampling of arterial blood drawn from the radial artery assessed the time-course of the arterial tissue radioactivity. This is represented by the blue curve on Figure 4. The corrected PET counts are drawn in pink. Thus, the PET counter detects the first activity at the third frame (20 seconds delay), whereas the arterial blood sampling counter perceives it 10-15 seconds later, because of the longer distance (~2x) for the blood to arrive to the arterial counter. We measured the arterial activity until 30 seconds after the end of the scan.

Figure 5 represents an example of a quantitative flow map from a $H_2{}^{15}O$ PET. The red spots correspond to regions with a high regional CBF. For example, on the two first slices (up and left), the petrosal part of the two internal carotid arteries can be recognized.

The difference of $rCBF_{gray}$ on day 3 and day 1 is demonstrated in Table 2. There was a 14 % decrease of $rCBF_{gray}$ (8.047 ml/min/100gr; SD=4.88) in the group that stopped smoking and only a minimal change in the group that continued smoking (0.967 ml/min/100gr; SD=3.46). The difference between the groups was highly significant (p=0.00015).

Table 2 illustrates also the significant difference ($p<0.001$) of the expired CO before the PET on day 1 and 3. Obviously the group who stopped smoking had a very significant decrease of CO values (–90,36 %; SD=18.84). However, the group who continued smoking showed an 11.03 % decrease of CO values (SD=5.46). Compared to stable CO values, this CO decrease is not significant (p=0.1).

$PaCO_2$ was measured before every scan. On day 3 the group A showed slight $paCO_2$ decrease (–3.35 %; SD=5.46). In contrast group B showed out a minimal decrease of $paCO_2$ (–1.10 %; SD=6.40). These minimal changes were not significant (p=0.59).

The blood pressure was measured after every scan. The variation of systolic blood pressure between day 1 and day 3 was in group A +0.6 mmHg (SD=9.39) and –0.2 mmHg (SD=6.39) in group B. On the other hand, the diastolic values of the blood pressure showed variations of +0.7 mmHg (SD=8.02) for the group A and of –2.5 mmHg (SD=4,86) for the group B. These changes in the blood pressures were only minimal and not significant (systolic BP p=0.65; diastolic BP p=0.12).

The craving intensity was measured with the Tiffany questionnaire (QSU-Brief), before the PET on day 1 and day 3. The total urge score was calculated by the mean of the 10 items. Figure 6 represents the difference of craving intensities on day 3 compared with day 1 (baseline). Group A showed a significant increase on day 3 (+1.35 points; SD=1.24) and group B demonstrated a slight decrease (–0.38 points; SD=0.75).

The result of statistical parametric mapping (SPM) searching for excessive changes in rCBF is shown in Figure 7 and Figure 8.
Figure 7 demonstrates areas that show an excessive decrease of blood flow from day 1 to day 3, that is where the decrease is significantly (T>5) higher than the general decrease of 14 %. The analysis reveals one area in the left insular cortex. A ROI analysis demonstrated that the decrease in this area was -19.4 % (Figure 7). The color spots at the surface of the brain correspond to artifacts.
Figure 6 reveals areas that show the smallest gray matter decrease with T>5 from day 1 to day 3 in the group that stopped smoking. The analysis identified the cerebellum. The ROI analysis demonstrates that the change in the cerebellum was only -2.9 % (Figure 8).

Figure 9 summarizes the changes in regional cerebral blood flow. First is represented a global gray matter CBF decrease in the group who stopped smoking and a minor CBF increase in the group who continued to smoke. In group A, all the analyzed regions (blue) illustrated a drop of rCBF. There was

a clear discrepancy between the cerebellum (–2.5 %) that showed no significant change between day 1 and day 3 (p=0.61) and the other investigated areas, which all demonstrate a significant decrease (range: –9.3 % to –19.6 %). In group B, no significant regional difference was found between day 1 and day 3.

Effects of experimental conditions were explored with repeated-measures analyses of variance (ANOVA) for the global blood flow and the cerebellum. The hypothesis with no ROI effect was significant $F_{(1,18)}$=7.43; p=0.014, as well as the hypothesis with no ROI*Group effect $F_{(1,18)}$=4.63; p=0.045. The repeated measure ANOVAs demonstrated an effect of experimental condition, when compared between group A and B and Day 1 and 3, on the global blood flow $F_{(1,18)}$=22.67; p=0.0002, but not on the cerebellar blood flow $F_{(1,18)}$=0.81; p=0.379.

5 DISCUSSION

The major result of this study is the demonstration of a significant decrease of rCBF in smokers who interrupt nicotine intake. The decrease seems to be more or less homogeneous in the gray matter of the neocortex and is in the order of 14%. Almost no change is noted in the cerebellum. The control parameters ($paCO_2$ and blood pressure) did not show any significant variations for both groups, while the parameters controlling the smoking cessation (CO and QSU-Brief) showed a significant change for group A, but not for group B. The 11% decrease of CO values in the control group could be explained by the in-hospital condition and by the simultaneous presence of another volunteer who often took place in the non-smoking group (according to our randomization table).

The CBF decrease seen in our nicotine withdrawn subjects could correspond to the interruption of the vasodilative effect of nicotine. Every time that a smoker smokes, his plasma nicotine concentration and his CBF increase. Every time that a smoker does not smoke, his plasma nicotine concentration and his CBF slowly decrease, until the next cigarette. In the situation of total abstinence, the CBF would return to original values, but under the toxic action of nicotine on the arteries, the CBF of smokers progressively decrease over the years (~10%). Consequently the CBF values of the nicotine withdrawn smoker in the acute phase lays under the CBF values of the non-smoker and under the values of the smoking smoker. These lower values lasts months to years, until the ex-smoker partially recovers his original CBF.

Previous studies

Volkow[30] (1999) reported a study where no CBF change was found after 4 hours of smoking cessation (short-term abstinence) and another one with a decrease of rCBF in the hippocampus bilaterally (after 4 to 6 hours of nicotine deprivation). The aim of these 2 studies was to demonstrate a difference between the rCBF in smokers, compared with non-smokers and primarily not the craving effects. Although the subject can endure some withdrawal symptoms after 4 to 6 hours smoking cessation, these 2 studies did not show a clear demonstration of a complete nicotine withdrawal syndrome.

Conversely, 2 studies evaluated the CBF of long-term abstinent smokers. Rogers et al.[31] (1985) showed in 11 abstinent smokers (from 1 to 12 months of smoking abstinence) a significant CBF recovery of 1 ml/min/100gr every month. Yamashita et al.[32] (2000) showed in six smokers an improvement of the rCBF, only after 4 to 6 years of tobacco interruption. After months of smoking abstinence, craving mostly persists and the CBF return slowly to his original values.

To our knowledge, there is no study evaluating the rCBF in smokers at a high craving state (32 hours smoking abstinence in our study). At this time, the nicotine withdrawal somatic symptoms are present and the relapse rate is high.

5.1 The nAChR hypothesis

In a normal brain the nAChR density is high in some regions (thalamus, caudate nucleus, frontal and parietal cortex) and low in temporal and occipital cortex, hippocampus and cerebellum.

In the particular case of the cigarette smoker, the global density of nAChR is higher than in a non-smoker's brain (up-regulation). Secondly they are mostly in a desensitized or inactive state. Human post-mortem studies have found a significantly higher density of nicotinic receptors labeled by [^{3}H]-nicotine in brain samples from smokers compared with non-smokers in the gyrus rectus of the frontal lobe, the hippocampus, the cerebellum and the thalamus[33].

To understand the density variation of the nAChR in a brain during nicotine withdrawal syndrome, Pietilä et al. (1998)[34] showed in mice, chronically treated with nicotine that the receptor density decrease and return to control level 7 days after nicotine withdrawal.
The up-regulation of nicotinic receptors in mice has previously been reported by Collins et al.[35] (1988) to return to control levels within 6–8 days after cessation of chronic nicotine treatment. In rats, the up-regulation of the

nAChR returns to normal level after 15-20 days after nicotine cessation. (Collins 1990).
In 2 baboons after chronic nicotine treatment, Kassiou et al.[36] (2001) demonstrated a full nAChR down-regulation in 3 regions (frontal lobe, thalamus and cerebellum) after 5 weeks nicotine withdrawal.
In human brains, Breese et al.[37] (1997) demonstrated in thalamus and hippocampus the recovery of the nAChR up-regulation by smokers, who stopped smoking at least 2 months prior to death. The other gray matter regions were not analyzed.
Even with the lack of data in the literature concerning the other gray matter regions of the human brain, we could postulate that in these, the up-regulation recovery lasts at least some weeks. It seems improbable that this nAChR down-regulation after nicotine cessation could explain the CBF decrease seen in our subjects, because they were enduring a nicotine withdrawal of 36 hours long. But during that time, the desensitized nAChRs partially re-sensitize. This re-sensitization of the nAChR can lead to an excess of receptive receptors, but without the agonist nicotine. This hyper-excitability state at cholinergic synapses plays probably a role in the development of the craving symptoms. The question remains if this hyper-excitability plays a role in the CBF decrease of our subjects.

5.2 The NBM hypothesis

Some studies on animals demonstrated the participation of the nucleus basalis of Meynert (NBM) to cause a nicotine-induced cholinergic neuronal vasodilation. Meynert described the anatomy of these magnocellular neurons at the base of the brain in 1872. The nucleus basalis is a part of the basal forebrain, which is a group of structures (NBM, septal area and the diagonal band of Broca) that are just rostral to the diencephalon. The basal forebrain projects dense cholinergic pathways to the surrounding structures, as well as the NBM which projects also to most regions of the neocortex.

Uchida and Sato (1997) measured the CBF responses to an intravenous nicotine administration in rats under different conditions. Some had the NBM electrically injured on both sides, some received mecamylamine (MEC), a

blood brain barrier permeable nicotinic receptors antagonist. Others were given atropine (muscarinic cholinergic antagonist) or hexamethonium. The nicotine-induced CBF increase was largely reduced by these three nicotine antagonists. The rats with NBM lesions had comparable CBF reductions. The authors concluded that the nicotine-induced cortical vasodilation was mediated by activation of the nicotinic receptors in the NBM[38].

In addition Linville et al. (1991, 1993)[39,40] provoked CBF increases in rats by electrical micro-stimulation of the basal forebrain. They also demonstrated that nicotine microinjections into the basal forebrain elicited profound increases in cortical CBF, whereas similar injections into the cerebral cortex were without effect suggesting that nicotine receptors mediating CBF increases are localized to the basal forebrain.

Nicotine is thought--at least in rats--to increase the CBF partially through the activation of the neurons situated in the NBM, projecting in most part of the cortex, but not to the cerebellum; the remaining part of activation is composed by local metabolites and by others projecting neurons.

In the human brain, the role of the NBM under nicotine and under a nicotine withdrawal situation is still unclear. But if the human NBM functions like the NBM of the rat, we could suggest that with every cigarette, nicotine stimulates the NBM producing a CBF increase as long as the nicotine plasma levels are high. At nicotine withdrawal, the nAChR of the NBM are not anymore activated and thus the CBF decrease.

5.3 Other Considerations

Cerebellum

The cerebellum of our subjects, withdrawn from nicotine did not show any significant CBF changes, in comparison of the gray matter of the neocortex. The cerebellum has in contrast with other gray matter brain areas, a lower nAChR density and a higher subunit polymorphism. Some authors reported a significant nAChR up-regulation in the cerebellum after chronic nicotine treatment in humans, but not in mice.

As the cerebellum of our subjects has also up-regulated nAChRs, we should have observed a similar CBF decrease between the cerebellum and the neocortex. This makes the nAChRs hypothesis unlikely.
In opposition, the absence of a cholinergic innervation from the nucleus basalis of Meynert to the cerebellum[41,42] could explain the lack of CBF decrease observed in the cerebellum.
In addition, the cerebellum reacts under an acute nicotine administration with a CBF decrease, as reported by Ghatan et al (1998) and Nagata et al. (1998). This might be explained by a steal-effect from the cerebellum to the neocortex, when the latter increase his CBF under acute nicotine administration.

Left Insula

Once the global change in rCBF was established, statistical parametric mapping SPM99 was used to search for focal areas that might deviate from the global range. This efficient tool revealed one small focus in the left insular area where the decrease of rCBF significantly exceeded the mean decrease (14%) of whole brain gray matter. In this area, the decrease was approximately 19%.
Projections from the NMB to the insular cortex have been documented, as well as from the insular cortex to the NMB[43]. However the physiological meaning of this finding remains at present not clear.

6 CONCLUSION

To our knowledge, this is the first study evaluating the rCBF of smokers in the acute phase of nicotine withdrawal. The major finding of this study is a 14% decrease of CBF in the gray matter, except in the cerebellum where no significant change was noted.

During nicotine withdrawal by smokers, it has been demonstrated that down-regulation of up-regulated nAChRs lasts weeks or months, indicating that nicotinic receptor density is probably not the factor involved in the CBF decrease.

Moreover, the forebrain contains a neuronal cholinergic pathway, originating from the nucleus basalis of Meynert and projecting to the majority of the regions of the cortex. Its cholinergic activation is known to produce a significant CBF increase in the rat. One may therefore speculate, that a deactivation of the NBM responsible for the decrease in CBF shown in this study. This is further supported by the fact that no change was observed in the cerebellum, which do not received projections from the NBM.

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8. TABLES AND FIGURES

Tiffany Short Form Craving/Urge to Smoke Questionnaire

Indicate how much you agree or disagree with each of the following statements by placing a single checkmark (like this X) along each line between strongly disagree and strongly agree. The closer you place you place your checkmark to one end or the other indicates the strength of your disagreement or agreement. Please complete every item.

1. I have a desire for a cigarette right now
STRONGLY DISAGREE __:__:__:__:__:__:__: STRONGLY AGREE

2. Nothing would be better than smoking a cigarette right now
STRONGLY DISAGREE __:__:__:__:__:__:__: STRONGLY AGREE

3. If it were possible, I probably would smoke now
STRONGLY DISAGREE __:__:__:__:__:__:__: STRONGLY AGREE

4. I could control things better right now if I could smoke
STRONGLY DISAGREE __:__:__:__:__:__:__: STRONGLY AGREE

5. All I want right now is a cigarette
STRONGLY DISAGREE __:__:__:__:__:__:__: STRONGLY AGREE

6. I have an urge for a cigarette
STRONGLY DISAGREE __:__:__:__:__:__:__: STRONGLY AGREE

7. A cigarette would taste good now
STRONGLY DISAGREE __:__:__:__:__:__:__: STRONGLY AGREE

8. I would do almost anything for a cigarette now
STRONGLY DISAGREE __:__:__:__:__:__:__: STRONGLY AGREE

9. Smoking would make me less depressed
STRONGLY DISAGREE __:__:__:__:__:__:__: STRONGLY AGREE

10. I am going to smoke as soon as possible
STRONGLY DISAGREE __:__:__:__:__:__:__: STRONGLY AGREE

Figure 1: Tiffany short form craving questionnaire.

FAGERSTROM TOLERANCE QUESTIONNAIRE

Date of assessment ___/___/___ Time ___/___
Please read each question below. √ only one box for each question that best describes your response.

1. How soon after you wake up do you smoke your first cigarette?

within 30 minutes ☐ (1)
after 30 minutes ☐ (0)

2. Do you find it difficult to refrain from smoking in places where it is forbidden, eg. in church, at the library, at the movies etc?

yes ☐ (1)
no ☐ (0)

3. Which cigarette would you hate to most to give up?

the first one in the morning ☐ (1)
any other ☐ (0)

4. How many cigarettes per day do you smoke?

14 or less ☐ (0)
15-25 ☐ (1)
26 or more ☐ (2)

5. Do you smoke more frequently during the first hours after awaking than during the rest of the day?

yes ☐ (1)
no ☐ (0)

6. Do you smoke even when you are so ill that you are in bed most of the day?

yes ☐ (1)
no ☐ (0)

7. What is the nicotine level of your usual brand of cigarette?

0.5 mg or less ☐ (0)
0.6 - 1.1 mg ☐ (1)

1.2 mg or more ☐ (2)

8. Do you inhale?

Never ☐ (0)

Sometimes ☐ (1)

Always ☐ (2)

Figure 2: the Fagerström tolerance questionnaire estimates the level of dependence of smokers

Table 1 : Volunteer Data

VOLUNTEER DATA		range			mean			SD		
Group		All	A	B	All	A	B	All	A	B
Age	*years*	**35_61**	35_45	35_61	**44.1**	40.2	48.0	**7.7**	3.8	8.8
cigarettes/day	*unit*	**20_80**	20_80	20_50	**32.0**	33.8	30.3	**15.7**	20.0	10.7
smoker since	*years*	**15_40**	15_30	17_40	**25.8**	23.7	27.9	**6.8**	5.5	7.5
Fagerström Quest.	*points*	**7_10**	7_10	7_10	**8.1**	7.9	8.3	**1.1**	1.0	1.2
Coffee/day	*unit*	**1_6**	2_6	1_6	**4.1**	4.5	3.8	**1.8**	1.7	1.8
Alcool/day	*unit*	**0_3**	0_2	0_3	**1.0**	0.8	1.1	**0.9**	0.9	1.0

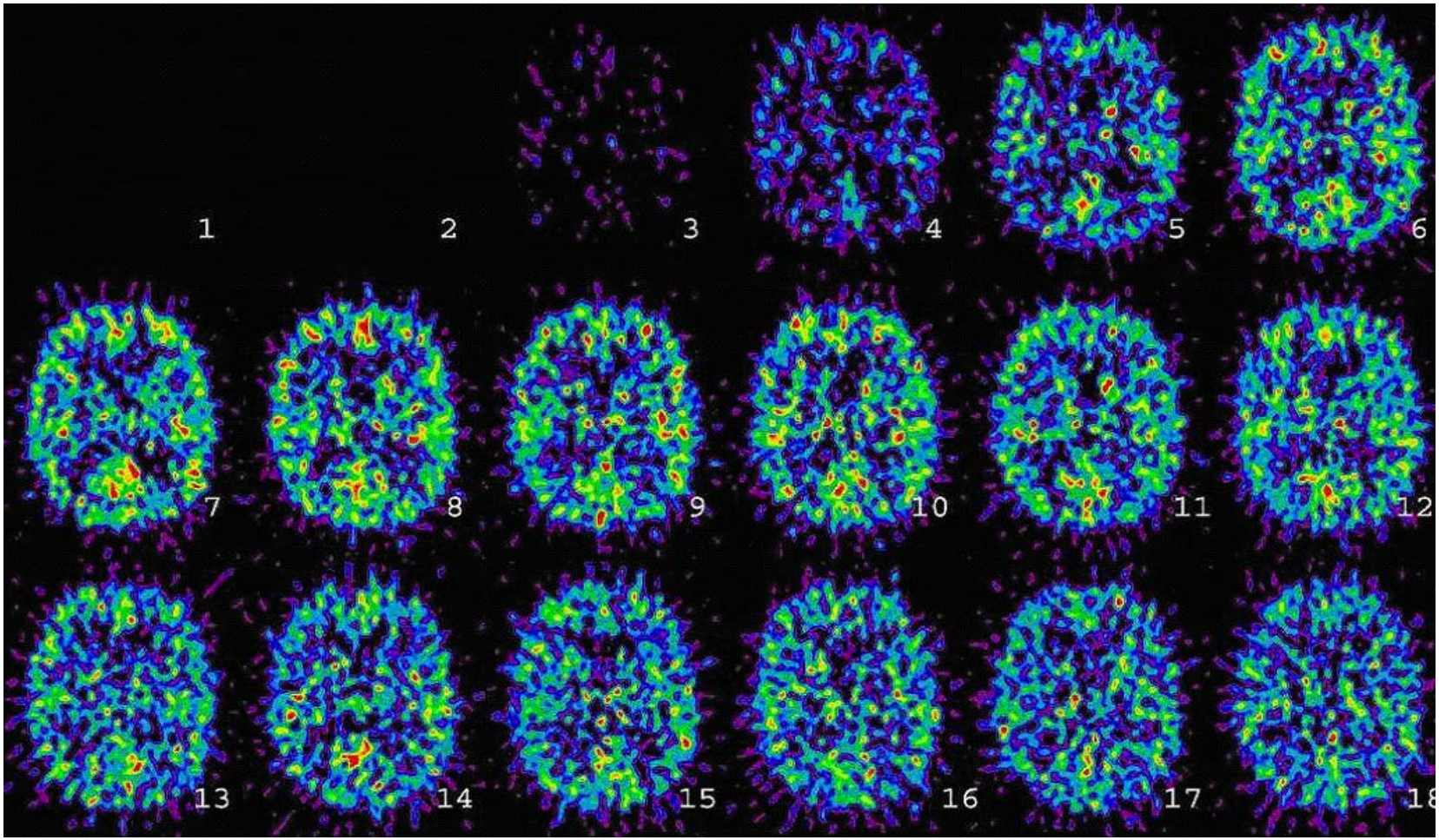

Figure 3: Time course of $H_2{}^{15}O$ over 18x10 seconds. Each image constitutes a frame of 10 seconds duration.

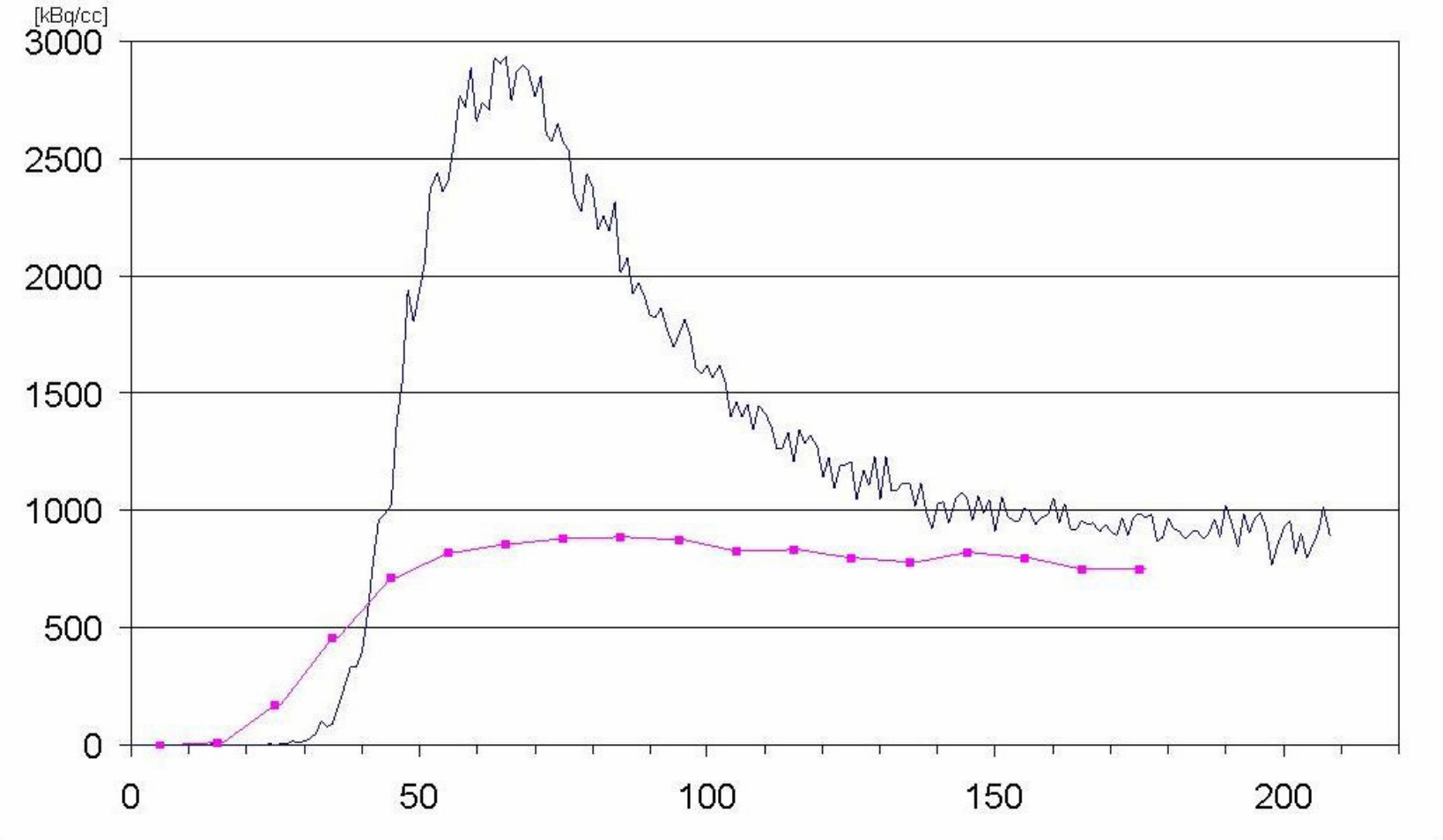

Figure 4: Time course of $H_2{}^{15}O$ concentration in arterial whole blood (blue curve) and tissue (pink curve).

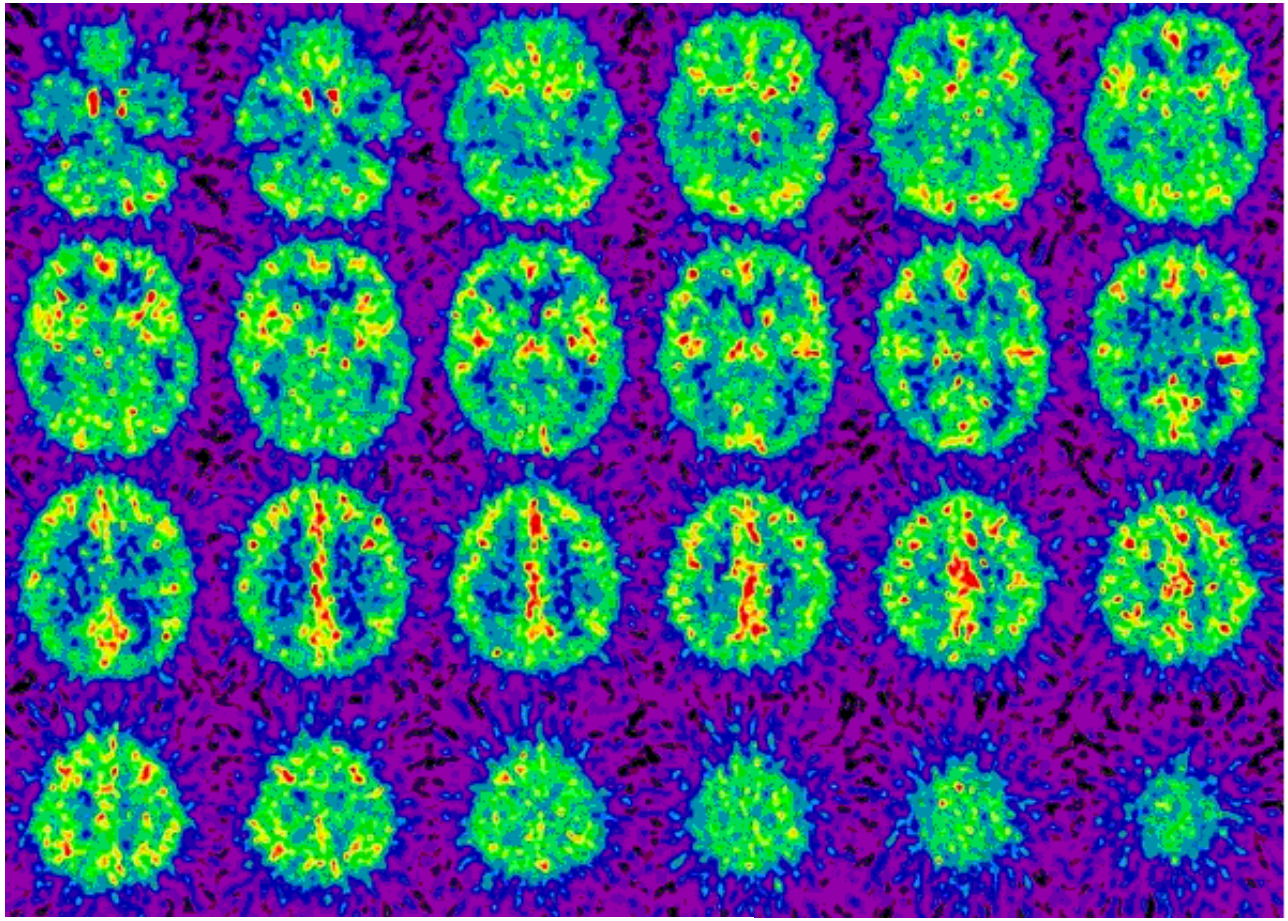

Figure 5: Example of a quantitative flow map derived from $H_2{}^{15}O$ PET.

Subject Number	Group	rCBF [ml/100g/min] day1	rCBF [ml/100g/min] day3	rCBF [ml/100g/min] day3-day1	CO [ppm] day1	CO [ppm] day3	CO [ppm] day3-day1	paCO2 [kPa] day1	paCO2 [kPa] day3	paCO2 [kPa] day3-day1
2	B	53.50875	56.53588	3.027	27	24	-11.11%	5.86	5.17	-11.77%
3	B	55.47985	49.15813	-6.322	15	14	-6.67%	5.41	5.44	0.62%
5	B	57.68959	56.93540	-0.754	28	26	-7.14%	5.73	5.51	-3.98%
8	B	45.52751	46.16094	0.633	21	20	-4.76%	5.24	5.01	-4.33%
9	B	42.80260	43.07138	0.269	37	49	32.43%	5.10	5.05	-1.05%
11	B	56.96181	60.82729	3.865	29	19	-34.48%	5.41	5.06	-6.47%
12	B	60.92357	58.38767	-2.536	37	35	-5.41%	5.76	5.77	0.29%
15	B	57.56074	60.70233	3.142	39	27	-30.77%	5.52	5.91	6.94%
18	B	60.40047	63.86334	3.463	53	39	-26.42%	6.10	5.49	-10.10%
19	B	41.42280	46.30161	4.879	25	21	-16.00%	5.75	5.54	-3.60%
1	A	55.92850	51.60635	-4.322	25	1	-96.00%	5.23	5.35	2.23%
4	A	52.98379	46.41460	-6.569	41	7	-82.93%	5.61	5.77	2.85%
6	A	50.43000	43.63333	-6.797	17	3	-82.35%	5.80	5.83	0.63%
7	A	48.76718	47.36337	-1.404	20	1	-95.00%	5.49	5.58	1.70%
10	A	55.92343	43.83372	-12.090	12	2	-83.33%	5.63	5.72	1.60%
13	A	52.59782	47.06651	-5.531	23	1	-95.65%	5.72	5.74	0.41%
14	A	59.54954	50.05053	-9.499	16	1	-93.75%	5.62	5.43	-3.44%
16	A	57.02366	42.21361	-14.810	31	3	-90.32%	6.41	5.23	-18.36%
17	A	59.03077	43.11035	-15.920	27	2	-92.59%	5.65	5.82	3.13%
20	A	68.55606	65.02726	-3.529	36	3	-91.67%	5.76	5.65	-1.80%
	Mean gr. B	53.22777	54.19440	**0.967**			**-11.03%**			**-3.35%**
	SD gr. B	7.27	7.35	3.46			18.84			5.46
	Mean gr. A	56.07908	48.03196	**-8.047**			**-90.36%**			**-1.10%**
	SD gr. A	5.62	6.71	4.88			5.46			6.40

Table 2: Whole brain gray matter blood flow, expired CO and $paCO_2$ at the time of PET measurements.

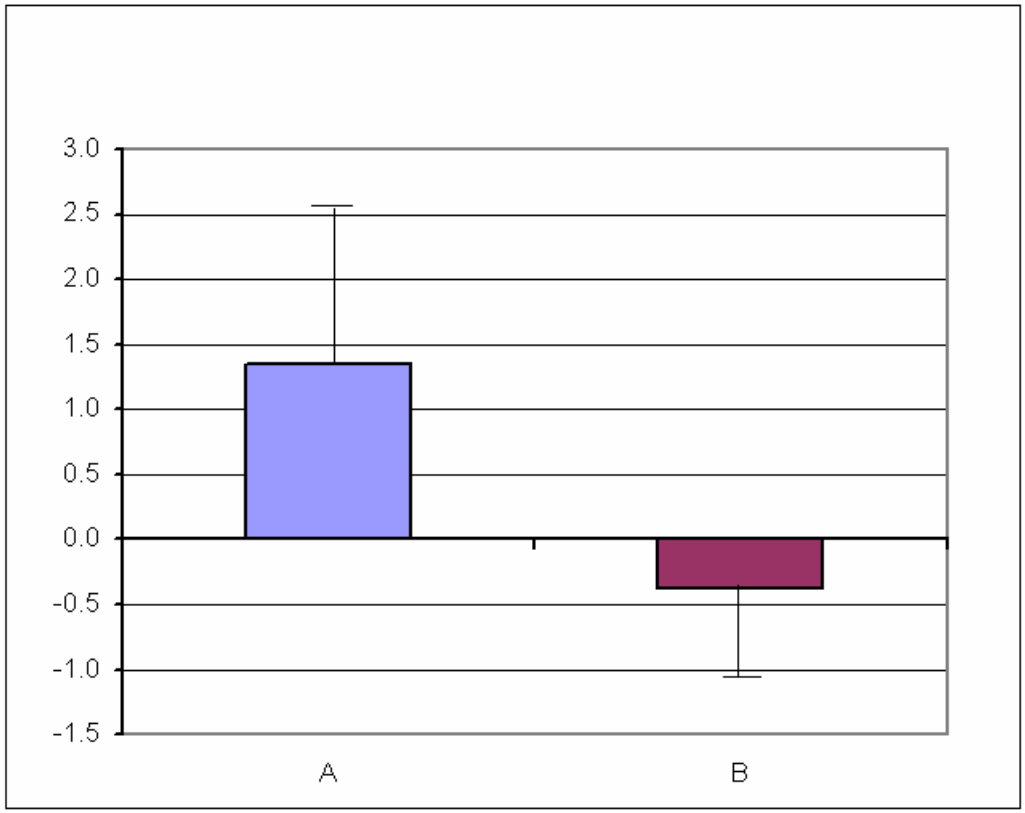

Figure 6: total urge score changes of the Tiffany questionnaire (QSU-Brief). Shown is the score difference of day 3 from day 1 (baseline) for both groups. The total urge score range from 1-7.

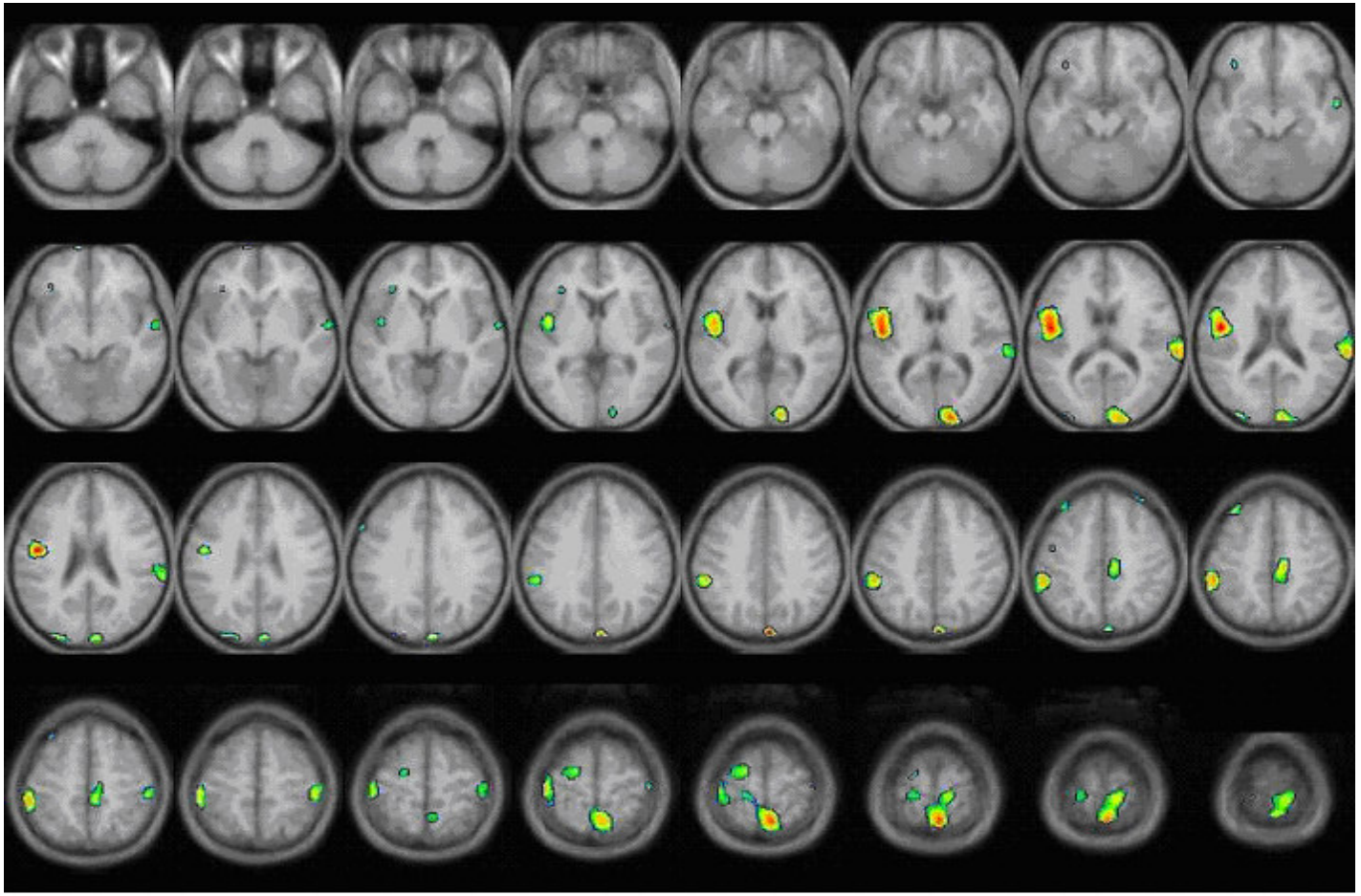

Figure 7: Statistical parametric mapping, in colour are areas with T>5 for the difference day1-day3 in the group that stopped smoking, indicating an excessive decrease in rCBF (greater than global decrease).

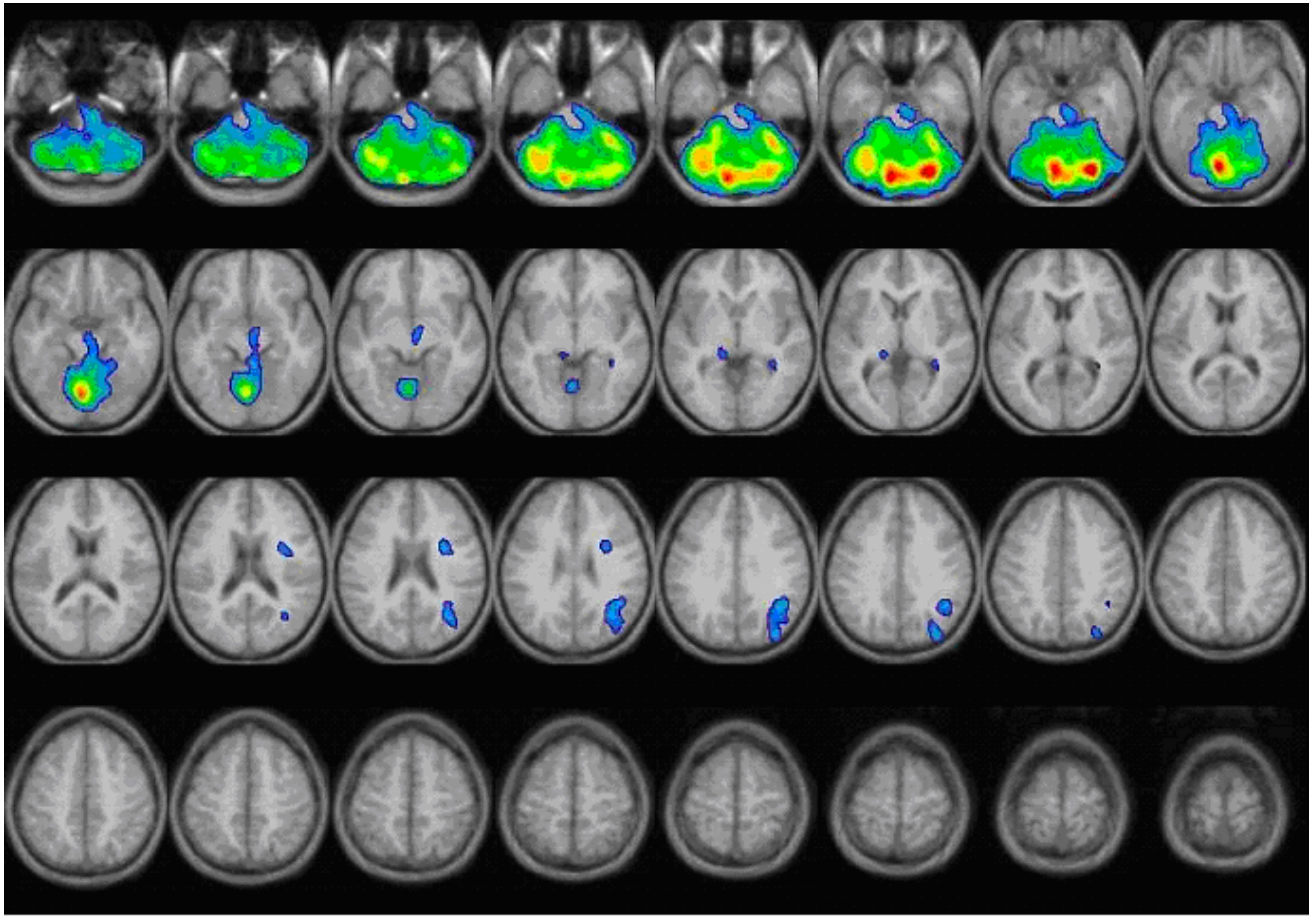

Figure 8: Statistical parametric mapping, in colour are areas with T>5 for the difference day3-day1 in the group that stopped smoking, indicating no significant change from baseline. In red are areas, which decreased the fewest.

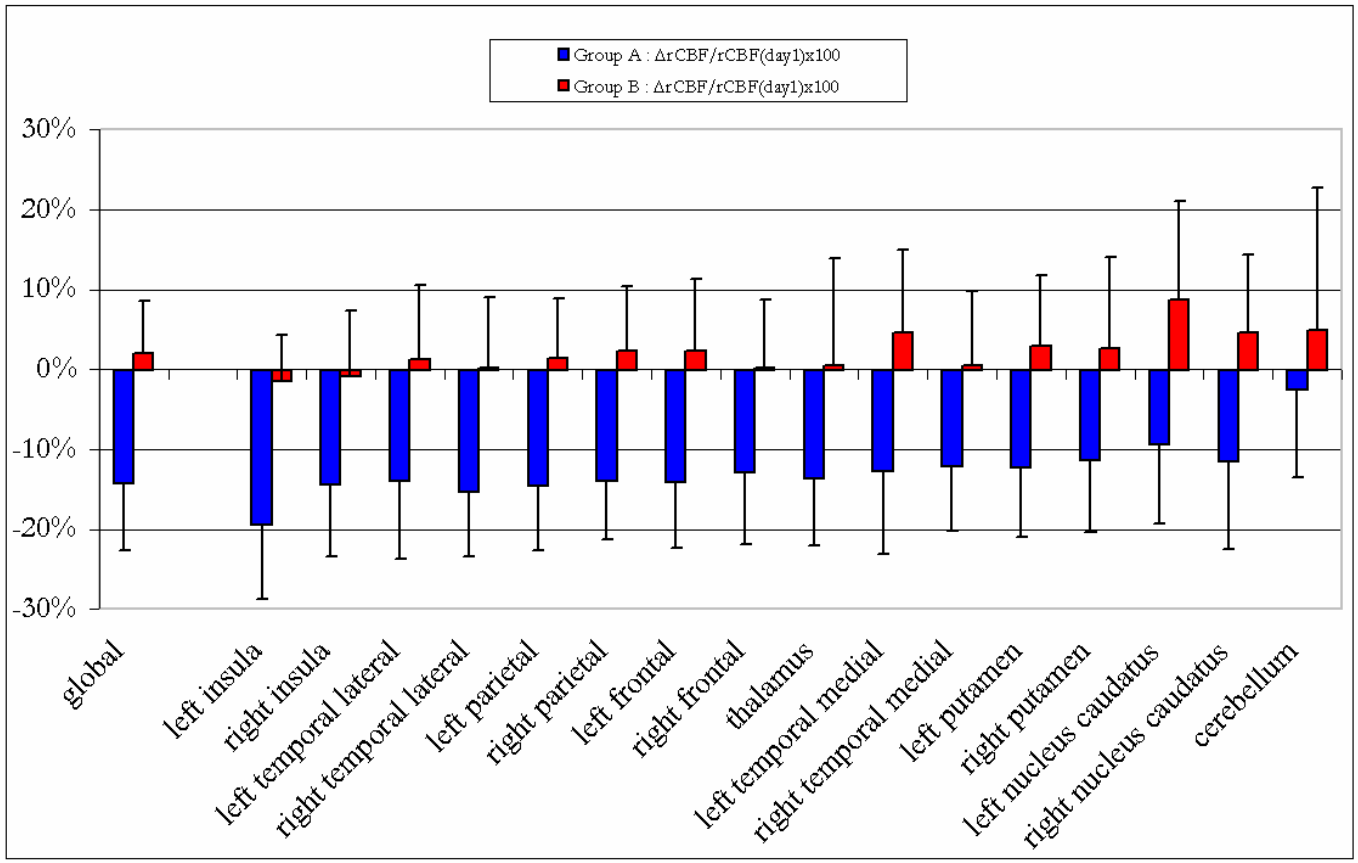

Figure 9: Regional blood flow changes. Shown is the percentage difference from baseline for both groups.

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